REMARKS

Claims 27-69 are pending.

The Specification has been responsively amended to reflect proper use of trademarks.

The Examiner's new matter rejection is acknowledged, and applicants have respectfully traversed this particular basis of rejection, and have provided supporting literature references.

The Examiner's rejection under 35 U.S.C. § 112 ¶2 for indefiniteness is acknowledged, and applicants have responsively amended the appropriate independent claims to further clarify the subject matter.

The Examiner's rejections under 35 U.S.C. §§ 102 and 103(a) for anticipation and obviousness, respectively are acknowledged, and applicants have respectfully traversed these particular bases of rejection.

The Examiner's rejections under obviousness-type double patenting are acknowledged, and applicants are prepared to timely file a Terminal Disclaimer.

No new matter has been added.

FORMALITIES

The Specification has been amended to reflect proper use of trademarks and respective generic terminology in view of the Examiner's comments.

New Matter

Dependent claims 35, 37, 46, 48, 58 and 60 were rejected by the Examiner under 35 U.S.C. § 112 ¶1as containing new matter. Specifically, the Examiner asserts that the Specification does not describe "molecular beacon-type probes," or "scorpion-type primers" (*see* Office Action of 26 March 2003, at page 2, para 4).

Applicants respectfully traverse this rejection, based on support found in the originally-filed specification at, *inter alia*, page 8, lines 18-20, page 15, lines 24-26, and page 16, lines 7-19 (as previously expressed in applicants' Preliminary Amendment of 10 December 2001). The Specification teaches, *inter alia*, use of dual-label FRET probes, and teaches use of LightCycler®

technology. Hydrolysis probes (*e.g.*, dual label TaqMan® probes), Molecular Beacons (*see* Tygai & Kramer Nature Biotechnology 14:303-308, 1996, attached hereto) and intramolecular Scorpion-type probes are art recognized Dual label FRET probes, and as recognized in the art at the time of filing, also *standard* real-time LightCycler® fluorescence formats (*see e.g.*, for discussion and attached hereto: Foy & Parkes, *Clinical Chemistry* 47:990-1000, 2001; *referencing*, for Molecular Beacons, Tygai & Krame, *Nature Biotechnology* 14:303-308, 1996, teaching real-time applications of Molecular Beacons; and *referencing*, for Scorpion-type probes, Whitcombe et al., *Nat. Biotechnol.* 17:804-807, 1999, teaching real-time applications of Scorpion-type). A specification need not, and preferably does not teach what is already known to one skilled in the relevant art.

Applicants, therefore, respectfully request withdrawal of Examiner's New Matter rejection with respect to *dependent* claims 35, 37, 46, 48, 58 and 60. Dual-label FRET probes are specifically disclosed and taught, and Molecular Beacon- and Scorprion-type probes are standard art-recognized LightCycler® fluorescence formats (FRET formats).

35 U.S.C. § 112 ¶2 Rejections

Claims 27-69 were rejected by the Examiner under 35 U.S.C. § 112 ¶2 as being indefinite. Specifically, claims "27-59" [assume 27-69] are alleged to be indefinite over the recitation of "an amplification-mediated," in the absence of a "noun." Additionally, claims "27-59" [assume 27-69] are alleged to be indefinite over the recitation "or in a property thereof in relation to another probe or primer" (*see* Office Action of 26 March 2003, at page 3, para 5A).

Finally, claims 34-35, 45-46, 57-58, and 67 are alleged to improperly rely upon recitation of a trademark (*see* Office Action of 26 March 2003, at page 4, para 5B).

First, applicants have responsively amended *independent* claims 27, 38, 50 and 61 to recite "at least one of: an amplification-, or amplification product-mediated displacement or conformational change of the CpG-specific probe; or an amplification-, or amplification product-mediated displacement or conformational change of the probe in relation to another probe or a

primer" in place of "an amplification-mediated, or amplification product-mediated change in a property of the CpG-specific probe, or in a property thereof in relation to another probe or primer."

The Specification is replete with support for this amendment, particularly in the various teachings relating to the use of dual-label TaqMan® probes that, as known in the art and as taught herein, are either displaced or digested during amplification. Likewise, the specification teaches the use of "dual probe" variations of the FRET applications (*see e.g.*, Specification at page 8, lines 18-20). Dual probe real-time FRET applications, as known in the art, comprise displacement or conformation change of one probe relative to another (altering the distance between the respectively attached *fluorophore* and *quencher* moieties.

Accordingly, the ambiguity of the previously recited "property" has been removed, and "detecting" within the method has been clarified as based on amplification-, or amplification product-mediated displacement or conformational change of the probe or of the probe in relation to another probe or a primer.

Second, applicants have responsively amended *dependent* claims 34-35, 45-46, 57-58, and 67 to eliminate recitation of trademarks. Specifically, claims 34, 45, 57 and 67 have been amended to recite "real-time PCR" in place of "LightCycler®-type." Likewise, claims 35, 46, 58 and 67 have been amended to recite "dual-label hydrolysis probe" in place of "TaqMan®-type probe." Conforming amendments have been made to claims 33, 44 and 56, which now recite "dual-label hydrolysis probe" in place of "dual-label probe" to provide proper antecedent basis for "hydrolysis probe."

Support for these amendments is in the incorporated prior art, and is present throughout the originally filed Specification, where LightCycler® is taught and described as a real-time PCR-based platform, and where TaqMan®-type probes are shown to function, *inter alia*, as dual-label hydrolysis probes (*see e.g.*, in particular Figure 4).

Applicants, therefore, respectively request withdrawal of the Examiner's 35 U.S.C. § 112 ¶2-based rejection of claims 27-69, based on applicants' responsive amendments of *independent*

claims 27, 38, 50 and 61, and *dependent* claims 33-35, 44-46, 56-58, and 67 (all currently amended to conform with the above-described amendments).

35 U.S.C. § 102 Rejections

Claims 27-30, 36, 38-41 and 47 were rejected by the Examiner under 35 U.S.C. § 102 as being *anticipated* by Gonzalgo et al. (Nucleic Acid Research, 25:2529-2531, 1997).

Specifically, the Examiner alleges that Gonzalgo teaches: treatment of DNA with bisulfite to provide bisulfite-converted DNA; amplifying the converted DNA; electrophoresis to isolate PCR products; and incubation of the PCR products with Ms-SnuPE primers. Essentially, the Examiner alleges that the Ms-SnuPE primers function as a template 'probe' prior to extension, and further function as a "probe capable of distinguishing between methylated and unmethylated nucleid acid" (*see* Office Action of 26 March 2003, at page 5, para 6).

Applicants respectfully traverse this rejection, based on the fact that the Examiner has inadvertently misconstrued the teachings of Gonzalgo. Specifically, Gonzalgo teaches methylation-sensitive single nucleotide primer extension (Ms-SnuPE), which has, *inter alia*, the essential limitation of requiring a primer extension of the Ms-SnuPE primers ***before*** they can distinguish between methylated and unmethylated nucleic acid.

By contrast, in the present method the probe distinguishes between methylated and unmethylated nucleic acid *without* such extension. The Ms-SnuPE methods of Gonzalgo has no such probe element.

Furthermore, and even more significantly, detection in Ms-SnuPE is <u>not</u> by amplification-mediated displacement or conformational change in the probe (as in the instant claims). Rather, detection in Ms-SnuPE is mediated by a required post-amplification, post purification, post hybridization *extension* by <u>one</u> base. Ms-SnuPE is not a real-time assay. The instant methods are absolutely distinguishable, *inter alia*, by the detection step, in addition to the above-described fundamental difference in the nature of the probe. The teachings of Gonzalgo et al., do not anticipate the instant methods and kits.

Applicants, therefore, respectfully request withdrawal of the Examiner's 35 U.S.C. § 102 rejection of claims 27-30, 36, 38-41 and 47.

35 U.S.C. § 103 Rejections

Claims 61-65 and 68, were rejected by the Examiner under 35 U.S.C. § 103(a) as being unpatentable over Gonzalgo et al. (Nucleic Acid Research, 25:2529-2531, 1997) in view of Ahern (The Scientist, 9:20, 1995).

Briefly, as described above for the 35 U.S.C. § 102 rejection, the Examiner alleges that Gonzalgo teaches: treatment of DNA with bisulfite to provide bisulfite-converted DNA; amplifying the converted DNA; electrophoresis to isolate PCR products; and incubation of the PCR products with Ms-SnuPE primers. Essentially, the Examiner alleges that the Ms-SnuPE primers function as a template 'probe' prior to extension, and further function as a "probe capable of distinguishing between methylated and unmethylated nucleid acid" (*see* Office Action of 26 March 2003, at page 6, para 8).

Moreover, as further alleged by the Examiner, while Gonzalgo et al do not teach packaging reagents in to a kit, Ahern nonetheless does (*Id* at page 7).

Applicants respectfully traverse this rejection, based on the arguments described in detail above in response the Examiner's 35 U.S.C. § 102 rejection. Specifically, the Ms-SnuPE primers do not function as probes capable of distinguishing between methylated and unmethylated nucleic acids during amplification. Additionally and significantly, detection in Ms-SnuPE is not by amplification-mediated displacement or conformational change in the probe (as in the instant claims). Rather, detection in Ms-SnuPE is mediated by a required post-amplification, post purification, post hybridization *extension* by one base. Ms-SnuPE is not a real-time assay, and the instant methods are redily distinguished by the detection step alone, in addition to the fundamental difference in the nature of the probe. The teachings of Gonzalgo et al., alone or in combination as alleged, do not render obvious the instant methods and kits.

Applicants, therefore, respectfully request withdrawal of the Examiner's 35 U.S.C. § 103

rejection of claims 61-65 and 68 in view of the teachings of Gonzalgo and Ahern.

Claim 46, was rejected by the Examiner under 35 U.S.C. § 103(a) as being unpatentable over Gonzalgo et al. (Nucleic Acid Research, 25:2529-2531, 1997) in view of Whitcombe et al (US Pat. 6,270967, August 2001).

Specifically, the Examiner alleges that while Gonzalgo et al do not teach use of TaqMan probes, Whitcombe nonetheless does teach the use of such probes for real-time allele discrimination (*see* Office Action of 26 March 2003, at page 8, para 9).

Applicants respectfully traverse this rejection, based on the contentions twice described above. Furthermore, this rejection is particularly inappropriate, because a TaqMan probe, as taught in the instant invention and as known in the art, is a *hydrolysis* probe comprise both a fluorophore and a quencher (the fluorophore and quencher must be spatially separated (e.g., by hydrolysis) to give rise to enhance fluorescence). Extension of such a dual-labeled hydrolysis probe under the Ms-SnuPE format of Gonzalgo would not produce a fluorescence signal, much less one that could be related to the methylation status of the target nucleic acid, and certainly not one that could be so related in real-time.

Applicants, therefore contend that this rejection is most assuredly unsound, and respectfully request its withdrawal in view of applicants' claim 46.

Claims 42-45, were rejected by the Examiner under 35 U.S.C. § 103(a) as being unpatentable over Gonzalgo et al. (Nucleic Acid Research, 25:2529-2531, 1997) in view of Whittwer et al (US Pat. 6,140,054, October 2000).

Specifically, the Examiner alleges that while Gonzalgo et al do not teach use of FRET probes, Whittwer et al nonetheless do teach the use of such probes for real-time allele discrimination (*see* Office Action of 26 March 2003, at page 8, para 9).

Applicants respectfully traverse this rejection, based on the complete absence of any teaching or suggestion whatsoever in Gonzalgo, alone or in combination, to use FRET probes

capable of distinguishing between methylated and unmethylated DNA, by hybridization to bisulfite DNA. Neither the Ms-SnuPE primers of Gonzalgo, nor the FRET primers of Whittwer discriminate between methylated and unmethylated DNA.

Moreover, and significantly, the Whittwer method is only a ***quasi*** real-time method, because the method at its essence is based on monitoring fluorescence as a function of temperature to determine a "PCR product melting curve" (*see*, e.g., column 15, lines 55-58; column 16, lines 1-10). The "generated melting curve is then compared to the known melting curve for the mutant, normal or polymorphic sequence to determine the sequence of the target nucleic acid" (column 4, lines 14-17.

Significantly, in the Whittwer method, the FRET probe pair signal is completely abolished every time the PCR temperature is raised (dissociating and thus separating the FRET pair), and a melting curve must be determined and compared. The sensitivity of the assay gradually increases with repeated PCR cycles as the amount of amplificate (and thus product hybridization target) increase. The instant methods to not require determination of melting curves as do the claimed methods of Whittwer et al. For example, claim 1 of Whittwer recites, in (c), "monitoring the fluorescence as a function of temperature."

Applicants, therefore, respectfully request withdrawal of this rejection in view of applicants' claims 42-45.

Claims 27-30, 36, 50-53, 59, were rejected by the Examiner under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000).

Specifically, the Examiner alleges that Herman teaches: contacting "genomic DNA with bisulfite"; "amplifying the converted nucleic acid with primers that distinguish between methylated and unmethylated DNA such that at least one oligonuclotide probe is a CpG specific probe [**INCORRECT**]; and detecting based on amplification mediated change or property thereof in relation to another probe or primer" (*see* Office Action of 26 March 2003, at page 11, para 11).

Applicants respectfully and emphatically traverse this rejection because the Herman '704

method is MSP (methylation-specific PCR) that is described on page 5 lines 10-27 of the specification and differs in the choice of probes and kind of PCR reaction used. This rejection was already exahaustively and successfully rebutted in the issued parent of the instant application.

Briefly, the Herman '704 Patent process is called "MSP" in Herman '704 Patent (*see* column 3 line 55) and it is also called "MSP" in the present patent application. Figure 3 of the instant application best illustrates the differences between the present invention (called "MethyLight") and Herman's MSP. MSP requires that the PCR-amplified products are probed with probes that are not specific for methylated sites. In MSP, only the primers are specific for methylated sites. In MethyLight (present invention), by contrast, the probes must be specific for a methylated site. In Figure 3, the steps are illustrated where processes A and B have PCR primers that do not cover methylated sites but process B (inventive process) requires a probe that covers a methylated site. Further, in processes C and D, both have primers that cover methylated sites, but process C is MSP (Herman '704 Patent and its parent) because the probe does not cover a methylated site and process D is part of the present invention because the probe does cover a methylated site.

Specifically, Herman '704 describes the primers used in MSP as covering the "locus" in columns 6-7. The use of the term "locus" in Herman '704 refers to the hypermethylated site and CpG island. There is no disclosure or suggestion in Herman with regard to probes (see column 10) that a probe be specific to a methylated site or "locus" using Herman terminology.

Therefore, the difference between the present invention and MethyLight is the requirement that the probe be specific for the hypermethylated site and not the PCR primers. Accordingly, in view of this essential distinction, the present invention is neither anticipated by, nor obvious in view of the teachings of the Herman '704 Patent.

The Examiner alleges that Herman '146 "also teach the use of CpG-specific oligonucleotide probes" in columns 3-4 and claim 1. Applicants respectfully traverse this assertion. No where does Herman '146 disclose or suggest CpG-specific oligonucleotide **probes**. The essence of the Herman '146 MSP invention is CpG-specific **primers**, not probes. The

Herman '146 Patent is quite clear on this point. ("The **oligonucleotide primers** distinguish between modified methylated and nonmethylated nucleic acid." Herman '146 Patent abstract, emphasis added) Therefore, the Examiner's allegation is incorrect because it is methylation site specific oligonucleotide probes that is the essence of the present invention.

Likewise, the Examiner asserts (referring to column 9, lines 55-65 of Herman et al) that Herman suggests the use of ASO probes for sequencing. Whatever sequencing suggestion is being made here by Herman is with post-amplification, cloned DNA, and there is absolutely no teachings of suggestions in Herman et al, alone or in combination, that suggests use of CpG specific probes during amplification as a means to provide a real-time signal to distinguish methylated from unmethlyated DNA.

The present invention is patentable over both Herman patents because the claimed MethyLight process and kits require an oligonucleotide probe directed to a methylated site and not just primers directed to a methylated site. Accordingly, no *prima facie* showing of obviousness has been made.

Applicants have responsively amended *independent* claims 27, 38, 50 and 61 to recite "at least one of: an amplification-, or amplification product-mediated displacement or conformational change of the CpG-specific probe; or an amplification-, or amplification product-mediated displacement or conformational change of the probe in relation to another probe or a primer" in place of "an amplification-mediated, or amplification product-mediated change in a property of the CpG-specific probe, or in a property thereof in relation to another probe or primer."

Contrary to the Examiner's assertion, Herman et al. do not teach such amplification-mediated effects on the probe. In fact, the asserted Herman et al. reference does not teach: (i) the use of specific probes capable of distinguishing between methylated and unmethylated nucleic acid; (ii) PCR in the presence of a CpG-specific probe (*Id*); and (iii) detection of amplification by detection of amplification-mediated displacement or conformational effects on CpG specific probes (*Id*).

Applicants, therefore, respectfully request withdrawal of this rejection in view of

applicants' claims 27-30, 36, 50-53, 59 as amended herein.

Claims 31-34 and 54-57 were rejected by the Examiner under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied to claims 27-30, 36, 50-53 and 59 above, in view of Wittwer et al (U.S. Patent 6,140,054, Oct. 2000).

Specifically, the Examiner alleges that while Herman does not teach using FRET probes to detect allele specific differences, Wittwer et al nonetheless do (see Office Action of 26 March 2003 at page 13, para 12).

Additionally, claims 35 and 38 were rejected by the Examiner under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied to claims 27-30, 36, 50-53 and 59 above, in view of Witcombe et al (U.S. Patent 6,270,967, Aug. 2001).

Specifically, the Examiner alleges that while Herman does not teach using TaqMan probes to detect allele specific differences, Witcombe et al nonetheless do (*see* Office Action of 26 March 2003 at page 14, para 13).

Additionally, claims 61-65 were rejected by the Examiner under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied to claims 27-30, 36, 50-53 and 59 above, in view of Ahern et al (The Scientist, 9:20, July 1995).

Specifically, the Examiner alleges that while Herman does not teach packaging reagents to make kits, Ahern et al nonetheless do (*see* Office Action of 26 March 2003 at page 15, para 14).

Applicants respectfully traverse these three rejections, based on applicants' above respective arguments in relation to Gonzalgo in view of Wittwer, Whitcombe and Ahern, and based on applicants' above arguments rebutting the assertion under Herman et al., alone or in combination, under 35 U.S.C. § 103(a).

Applicants respectfully request withdrawal of these three rejections with respect to claims 31-34, 54-57, 35, 38 and 61-65.

Obviousness-type Double Patenting Rejection

The Examiners has rejected claims 27-32, 38-43, 50-55 and 61-67 under the judicially created doctrine of obviousness-type double patenting as being unpatentable in view of claims 1-26 of U.S. Patent No. 6,331,393 (December 18, 2001).

Applicants are fully prepared to timely file a Terminal Disclaimer upon notification of allowable subject matter.

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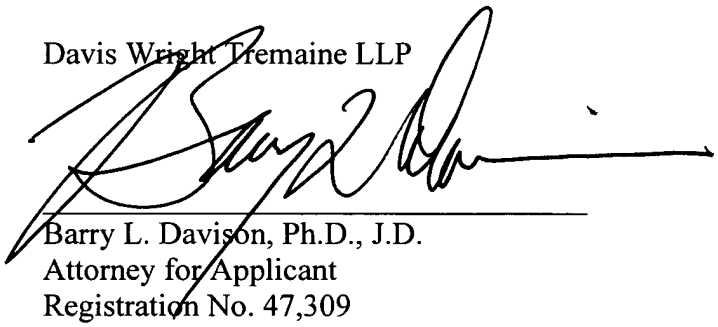
Applicants respectfully request consideration and allowance of all pending claims 27-69 of the present *continuation* application to provide for completion of claims supported by the original specification filed on 14 May 1999 (Issued as U.S. Patent 6,331,393).

No new matter has been added.

Entry of the Amendment is respectfully requested.

Respectfully submitted,

Davis Wright Tremaine LLP



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